INSERTION in FLS 1927 publication "The Report of a Nearly Pure Ancylostoma duodenale..." (paper 9), page 174, para. 1, line 11, between "peculiar" and "worm-species-content": "species of hookworms into regions occupied by people having a different"

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THE REPORT OF A NEARLY PURE *ANCYLOSTOMA DUODENALE* INFESTATION IN NATIVE SOUTH AMERICAN INDIANS AND A DISCUSSION OF ITS ETHNOLOGICAL SIGNIFICANCE.*

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Few contributions of the late Dr. S. T. Darling are of more fundamental interest than those touching the ethnological significance of various *Ancylostoma* : *Necator* ratios found in different parts of the world. What proved to be a posthumous publication of his (Darling, 1925) was concerned with some of the possibilities inherent in comparative helminthology in the solution of certain ethnological problems. Earlier (Darling, 1920) he had stated a principle as applicable to comparative hookworm infestations: "The races of mankind living in tropical and subtropical regions are infested with one or more species of hookworms. In the migrations of these peoples the immigrants have carried their peculiar worm-species-content, and by an examination of the intestinal worms of a people the geographical and ethnic origin of their hosts can, within certain narrow limits, be divined. I refer particularly to migrations within 35° N. and 30° S. latitudes, for when migrations are made into colder climates the hookworm infection is ultimately lost through inability of the embryos to persist during the phase of their life cycle spent in the soil."

Insert the missing line

Following the reported finding of *Ancylostoma duodenale* in appreciable numbers in Paraguay (Soper, 1924*a*, 1924*b*), Darling requested the writer to secure data regarding the species distribution of hookworms among Indians living in the more remote parts of that country where they would not easily become infected with *Necator americanus* from negroes. In this paper data will be presented on the species infestation of the Lengua Indians, living in the Gran Chaco Paraguayo,

* This paper is a contribution from the Department of Medical Zoology of the School of Hygiene and Public Health of the Johns Hopkins University, the data having been gathered in Paraguay under the auspices of the International Health Board of the Rockefeller Foundation.

Paper 9 has been considered of importance by workers in ethnology.

and attention will be called to the possible bearing of such data on the question of the origin of the Amerind race. Darling would have relished these data as another block in his world-map-mosaic of hookworm distribution and as an added bit of evidence in support of his theory.

In considering data of any kind from Paraguay, one must take account of the peculiar division of that country into two widely differing regions by the Paraguay river. That region lying on the left bank is a rolling, well watered, agricultural, grazing and forest district, about the size of the state of Missouri, which has supported a population of hundreds of thousands for many centuries. The original population consisted of Guarani Indians, a part of that great Tupi-Guarani stock which roamed from the Caribbean Sea to the Rio de la Plata, east of the Paraguay River. This Guarani stock has in great measure persisted and amalgamated with the Spanish, thus forming the Paraguayan race of today.

The contacts of this part of Paraguay with the outside world have been constant since the early part of the sixteenth century except for a period of about twenty years at the beginning of the nineteenth century, when, at the will of her first dictator, Paraguay became a hermit nation. From 1865 to 1870 the Triple Alliance waged a war, almost of extermination, against Paraguay. During and after this war many thousands of Brazilian troops, with a known heavy infestation of *Necator americanus*, were in the country and undoubtedly made their contribution to the hookworm picture of the survivors. The great apparent increase in hookworm disease in Paraguay following this war was so marked that local authorities attributed its existence to the presence of Brazilian troops, although from etymological considerations the existence of hookworms in Paraguay since early colonial times seems established (Soper, 1925, p. 417). Classification of hookworms from Paraguay east of the Paraguay River shows a ratio of *Ancylostoma* to *Necator* of 1 to 14 (Soper, 1925).

That part of Paraguay lying on the right bank of the Paraguay River and known as the Gran Chaco Paraguayo is an area of unknown extent and without definite boundaries to the north and west. Of geologically recent formation, the Chaco is thought to have been the bottom of an inland sea; low-lying and flat, it suffers from both floods and droughts. Quite close to the surface is found an impermeable layer of gumbo which prevents the absorption of the rainfall; thus the rain which falls, first floods the country and then drains off or

evaporates rapidly. Wells sunk through this impermeable layer usually give water too salty for use. Rivers one sees on maps of this region are wide swamps during wet seasons and but dry water courses during periods of drought.

The Chaco, then, at least that part visited by the writer, is ill-adapted to agriculture or to the support of a moderately dense aboriginal population depending on hunting and fishing; it has attracted neither the strong Guarani Indians nor the modern Paraguayans to take possession of it in force, and one can encounter today within a few days' ride of the Paraguay River Indian tribes whose contact with the outside world has been very limited.

The making of complete worm counts on aborigines is not easily accomplished, and the success of the work here reported is due to the full cooperation of all the members of the Anglican Mission at Makthlawaia, where they have had a station since 1908, although their work in this region dates back to 1889.

Makthlawaia, twenty-five or thirty leagues due west of Concepcion, is situated on a small island lying in the center of a circular lagoon; its population consists of a few white mission workers and about two hundred Indians, mostly of the Lengua tribe, living under very primitive conditions. Some gardening is attempted, but the principal occupation is cattle raising; some two or three thousand head of cattle are to be found in the herds belonging to the Indians.

The Lengua (Alarcon and Pittini, 1926) is not considered as a pure tribal type, but rather as a fusion of types. The Lenguas are reputed to have come to the Chaco from the west and to have furnished the ruling dynasties for several of the older tribes, *e.g.*, Sugines, Tozles and others. The Sanapaná, Carotuguis, Canaguatsan, Conamesma and some others are probably derived from the original Lenguas.*

Careful worm counts were made on complete forty-eight hour stools of 71 Indians at Makthlawaia following treatment with either 3 c.c. of oil of chenopodium or with 3.5 c.c. of a two by one mixture of carbon tetrachloride and oil of chenopodium with graduations of 1/18th per year of age for children. The group treated included eight women, seventeen boys, twenty-one girls and twenty-five men, five of whom were non-residents of the village and came from the region lying west of Makthlawaia. The data from these non-resident cases will be presented separately. The boys and girls studied ranged

* For further information regarding these tribes, the reader should consult Nordenskiöld (1919) and Grubb (Sceley, Service & Co.).

in age from 1 to 17 years of age; the child of one year of age treated proved negative and has been eliminated in the presentation of data.

Of seventy infected cases forty-one or 59 per cent. harboured *Necator americanus* and 70 or 100 per cent. harboured *Ancylostoma duodenale.* Of a total of 3,217 hookworms recovered but 228 or 7 per cent. were *Necator;* 2,989 or 93 per cent. were *Ancylostoma.*

TABLE 1.

Group.	Number in group.	Percent. with *Necator.*	Percent. of *Necator.*	Average no. *Necator.*	Average no. *Ancylostoma.*
Men.....	20	85.0	10.8	7.5	62.6
Women.....	8	87.5	4.4	3.4	73.7
Boys.....	17	52.9	4.0	1.4	32.6
Girls.....	20	40.0	4.5	1.3	27.6
Non-residents..	5	20.0	2.4	0.2	8.0

Table 1 shows the distribution of hookworm infection by species in the various groups treated. All individuals examined were infested with *Enterobius vermicularis;* no *Ascaris lumbricoides* nor *Trichuris trichiura* were found.

In considering the data from Paraguay, the author wishes to call attention to the fact that the dosage of anthelmintic used was sufficient to guarantee a high percentage (probably over 95 per cent.) of the number of hookworms harboured being expelled; further that *A. duodenale* is known to be remarkably more resistant to medication than is *Necator* and fails to be revealed in proper percentages in worm counts unless powerful doses have been administered.* The data from the Chaco are comparable with those reported for eastern Paraguay, since the same anthelmintics, the same technique and the same assistant were employed.

None of the cases treated in the Chaco had ever received treatment for hookworm disease, which precludes the possibility of previous medication having altered the *Ancylostoma* : *Necator* ratio. (Soper, 1924*b*.)

In Table 1, the *Ancylostoma* : *Necator* ratio is lower for men than for women and that for both boys and girls is approximately the same as for the women. This is interpreted to indicate that the village infestation is predominantly *Ancylostoma* and that *Necator* is

* This difference in resistance may help to explain the failure to report the existence of *Ancylostoma* in some regions where the Amerind race has fused with the white or negro races.

a newcomer, being brought in by the men, who make occasional trips to the river for supplies for the mission, and who have had during the past two years some contact with Paraguayan soldiers from east of the River, known to carry a heavy hookworm infestation with a low *Ancylostoma* : *Necator* ratio. (Soper, 1925.) That a pure *A. duodenale* infestation has been introduced into the Chaco in modern times is not probable, considering the difficulty with which *Necator* is becoming established, even with much greater contact with outside sources of infection than has ever occurred in the past.

It is not surprising to find that the men carry a lighter infestation than do the women, since much of the time they are working away from the village.

The percentage of each group infected with *Necator* seems to depend on the degree of total infestation of the group rather than on the degree of *Necator* infestation in the group; data based on percentage of group infested with a given species of hookworm do not give the same impression of relative importance of the two species in the group as do data based on the *Ancylostoma* : *Necator* ratio of the whole group. Hill and Earle (1924) found 25 per cent. of one group of 64 cases in Porto Rico infected with *Ancylostoma*, but only one per cent. of all hookworms present were of this species; in the present study 59 per cent. of the cases harboured *Necator*, although 93 per cent. of the hookworms found were *Ancylostoma*.

Smillie (1922) examined 34 Indians of the Terenos tribe, probably of the Tupi-Guarni race, living near Miranda, Matto Gross, Brazil, close to the Paraguay River and found an *Ancylostoma* : *Necator* ratio of 1 : 57. This ratio was enough higher than that found among native Brazilians, viz, 1 : 94 to suggest that the infestation had not been entirely derived from contact with Brazilians. Smillie describes these Indians (p. 58) as "pure-blooded semi-civilized Indians with no contact with whites or blacks although in the past there has been some communication with both Brazilians and Paraguayans," and later (p. 60) makes the significant statement, "They have had little contact with the whites though they fought in the Paraguayan War." This will explain the presence of *Necator americanus*, and from the present *Ancylostoma* : *Necator* ratio, it seems probable, without referring to historical records, that they spent the seven years of that war with the Brazilian rather than the Paraguayan troops.

Soper (1925) has reported finding an *Ancylostoma* : *Necator* ratio of 1 : 14 in Paraguay, east of the Paraguay River, and has explained this ratio as representing a heavy *Necator* infestation, derived partly

from negro slaves, but more largely from Brazilian troops, superimposed on a light *Ancylostoma* infestation present since early colonial days.

TABLE 2.

Group.	Location.	No. of cases.	Average *Ancylostoma* per case.	Average *Necator* per case.	*Ancylostoma*: *Necator* ratio.	Per cent of cases with *Ancylostoma*.	Per cent. of cases with *Necator*.
Brazilians...	Sao Paulo	112	0.4	52	1 : 194	10	?
Terenos Indians...	Matto Grosso	34	1.7	87	1 : 57	62	?
Paraguayans..	East of River Paraguay	419	13.4	184	1 : 14	78	98
Lengua Indians...	Chaco Paraguayo	70	42.7	3.3	13 : 1	100	59

The data in Table 2 summarize the available information regarding species distribution of hookworm in native Brazilians, Brazilian Indians, native Paraguayans and Chaco Indians. The *Ancylostoma* : *Necator* ratio in these different groups is progressively larger as one goes from zones of greater to zones of lesser contact with negroes.

The use of comparative parasitology in the study of species origins, of zoo-geography and of genetic relationships is relatively recent. It has been used, however, in helping solve many complex problems in a wide variety of fields. von Ihering (1902), working with helminths, used the distribution of entoparasites to help explain the genetic relationships of their hosts. Zschokke (1903) concluded from the relative importance of marine and fresh water entozoa in certain species of salmon that the original home of these species was marine, although spawning now occurs in fresh water. Zschokke (1904) again used the same method in explaining the distribution of certain cestodes of the marsupial mammals. Johnston (1912), working with trematodes of Australian frogs, called attention to the tendency of helminths to occur in faunal groups and stated the principle (p. 348) "that the helminths found parasitic in any particular class of host in a definite zoo-geographical region, find their nearest relatives not in that region in which themselves occur, but in the same class of host living in other zoo-geographical regions." Later (1914) Johnston extended his observations to include trematodes of mammals and birds and cestodes of marsupials, birds and amphibia.

Kellogg, by a study, first (1913) of bird hosts and later (1914) of mammals, showed that certain ectoparasites, although less completely isolated from the effects of changing environment than are entoparasites, show much less tendency to be modified than do their hosts and hence may be used to indicate relationships between certain groups of birds or animals having common ancestry.

Metcalf (1920, 1923) called attention to the importance of studies on *Opalina* parasites of frogs in determining the geographical distribution and former migrations of *Anura*, and shows that to explain the existing relationships, it is necessary to assume that there was previously a continental land connection between Australia and southern South America. The principle on which these conclusions are based is thus stated (1923, p. 356): "Having once met the conditions of parasitism and having undergone the initial modification to adapt them to the conditions of the new environment, some of their species are rather prone to persist without much further change, living as they do in a secluded, protected and remarkably uniform habitat."

Following a detailed description of present racial and geographical distributions of the two common species of hookworm, Darling (1920, p. 233) pointed out the possibility of either *Necator americanus* or *Ancylostoma duodenale* or both having been introduced into the American continent from Asia, Indonesia or Polynesia by voyagers or storm-tossed fishermen. He stated that migrations to America may have come (1) from Asia by way of Behring Straits, in which case, unless the temperature of that region in times past was warmer than it now is, hookworm infection would not be expected to persist; (2) from Asia or Indonesia by way of the Pacific in which case either or both *A. duodenale* and *N. americanus* might persist; or (3) from Polynesia by way of the Pacific in which case only *N. americanus* would be found. Darling concludes that (p. 232) "If certain tribes in America are found to be infected with *A. duodenale* as well as *Necator* this will suggest their having come to this continent via the sea from those countries in Asia where *A. duodenale* and *N. americanus* are found to be infecting the natives, i.e., Japan and China. . . . A careful hookworm survey of existing Indian tribes may disclose the presence of more than one primitive stock. . . ."

It is interesting to note that since the above was written, Hill and Earle (1924) have reported finding *A. duodenale* in Porto Rico, previously believed to have a pure *Necator* infestation, and Warren and Carr (1925) have found an appreciable infestation with *Ancylo-*

stoma among native Mexicans where the ethnic stocks are white and Amerind.

As stated above, the data from the Chaco are interpreted to indicate that *A. duodenale* came to this region with the Indian tribes, whereas *Necator* is only now being introduced from outside contacts; this would, according to Darling's theory indicate that the original Amerind stock originated in Asia or Indonesia, north of latitude 20° N. and migrated to America via the Pacific. Had migration been limited to Behring Straits, it must have occurred when the temperature of that region was more favorable to hookworm larvae than it now is, or it must have occurred from a region which was favorable to hookworm in Asia to a favorable zone in America in less than the maximum life span of the hookworm.

Hrdlička (1916), from ethnological data, considers all the Amerinds to be of mongoloid origin and believes that a triple invasion occurred via Behring Straits. Further helminthological studies on other isolated regions of the Americas, may reveal whether all immigrants carried the same species of worms or not and whether all invasions were by the same route.

It is of course possible that both *A. duodenale* and *Necator* came to America together and that adverse conditions of soil, climate, or mode of life, have blotted out *Necator* in certain districts or in certain tribes. All too little is known about the comparative life histories of *A. duodenale* and *Necator*. However, Smillie (1922, p. 52) stated: "We were able to prove readily that the factors of age, sex, soil, type of work and mode of life, though they greatly affect total infection with hookworms, do not affect the relative prevalence of *Ancylostoma* and *Necator*." Darling (1925, p. 332) believed that there is no difference between the adaptability of *Ancylostoma* and *Necator* to temperate, subtropical and tropical climates. Nevertheless, although it is true that under certain conditions the two species develop equally well, there is some evidence to suggest that under adverse conditions one species may survive and the other perish. Sawyer et al. (1923) reported finding an institutional infestation, predominantly *A. duodenale*, in a hospital lying outside the hookworm belt and drawing most of its patients from districts where *Necator* is the predominating species. Svensson (1925) found that there is a difference between the optimum developmental temperatures of *Necator* and *Ancylostoma* larvae and that larvae of *A. duodenale* live twice as long as do larvae of *Necator* at ice-box temperature.

The finding of a nearly pure infestation of *Ancylostoma duodenale*

among the Indians of the Chaco, must be explained in one of the following ways:

1. That the *Necator* element of a mixed *Ancylostoma* and *Necator* infestation has succumbed to conditions not sufficiently adverse to eliminate *A. duodenale.* If the mixed infection was of Asiatic origin, such adverse conditions may have been encountered en route to the Chaco or may exist in the Chaco; if it was of Paraguayan origin, i.e., from east of the Paraguay River, such adverse conditions must exist in the Chaco.

2. That the *Ancylostoma* infestation antedates that of *Necator*, in which case it must have come with the race from Asia, or have been introduced by the early Spanish conquistadores. In the light of local conditions and the past history of the Chaco, it seems most reasonable to believe that the *Ancylostoma* infestation of the Lengua Indians existed before the advent of the Spanish and that the *Necator* now present represents a very recent importation from east of the Paraguay River. Studies must be made in other regions before the possibility of an original mixed infestation of the Amerind stock with *Necator* can be ruled out.

Summary.

1. Seventy Lengua Indians living under semi-civilized conditions in the Paraguayan Chaco were found to have an *Ancylostoma* : *Necator* ratio of 13 to 1; this is by far the highest ratio reported for any group on the American continent.

2. The correlation of this finding with the species distribution of hookworms by races and geographical areas indicates that the Amerind race originated in Asia or Indonesia, north of latitude 20° N.; that migrations to America were either through the Pacific or via Behring Straits; if by the latter route special conditions (either climatic or of rapidity of migration) must have obtained at the time of migration.

3. Further studies on various isolated tribes in other parts of the Americas should be made; such studies may be expected to throw more light on the origin and migrations of the American Indian.

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